ABSTRACT

The amino acid sequence of a mutant which is obtained by introducing a novel mutation into a *Pseudonocardia thermophila* JCM3095-derived nitrile hydratase consisting of two types of heterogeneous subunits, and the base sequence of the gene are provided. The nitrile hydratase is modified by specifying the region to be modified in the stereostructure/amino acid sequence of the nitrile hydratase, and applying alteration such as substitution, insertion, deletion or the like, to the amino acids in the amino acid sequence which are corresponding to the amino acid residues forming the region. Also provided is a method for modifying an enzyme having a nitrile hydratase activity.

Please amend the paragraph at page 27, line 19, as follows:

The nitrile hydratase of the invention is obtained by introducing mutation into an enzyme having the nitrile hydratase activity, and for example, it is obtained by introducing mutation into *Pseudonocardia thermophila*-derived nitrile hydratase. Specifically, such nitrile hydratase fundamentally consisted of the amino acid sequences as set forth in SEQ ID NOs: 1 and 2 in the Sequence Listing, in which amino acid at one or more predetermined sites in the amino acid sequence is substituted by another amino acid. Thus, the invention comprises a nitrile hydratase having as its constituent the α-subunit represented by the sequence of 205 amino acids as set forth in SEQ ID NO: 1 in the Sequence Listing, in which at least one amino acid in the amino acid sequence is substituted by another amino acid; a nitrile hydratase having as its constituent the β-subunit represented by the sequence of ~~438 amino acids in total which constitute the β-subunit represented by the sequence of~~ 233 amino acids as set forth in SEQ ID NO: 2 in the Sequence Listing, in which at least one amino acid in the amino acid sequence is substituted by another amino acid; and a nitrile hydratase having both of the above-mentioned substitution cases as its constituent.

Please amend the paragraph at page 33, line 12, as follows:

[[(G-3)]] (G-5) a gene having the base sequence that codes for any one of the nitrile hydratases of the above-mentioned (A-1) to (B-4).

Please amend the paragraph at page 36, line 20, as follows:

[[(P-3)]] (P-5) a plasmid having the base sequences that code for several nitrile hydratases of the above-mentioned (A-1) to (B-4).

Please amend the paragraph at page 303, line 15, as follows:

Moreover, the plasmid was prepared from the cells by alkaline SDS extraction. Next, the base sequence of the nitrile hydratase gene was determined by the dideoxy chain termination method using a sequencing kit and an Autosequencer 373A manufactured by ABI. The results are shown in Table 98, in which it is known that the [[14th]] 148th Gly in the α-subunit in the wild nitrile hydratase was substituted with Asp and the 204th Val in the same was substituted with Arg.

Please amend Table 145 at page 463, as follows:

[Table 145]

Number	Mutated site	Change in amino acid sequence		Change in base sequence	
		Before mutation	After mutation	Before mutation	After mutation
No. 88c	168th position in β-subunit	Thr	[[acg]] Glu	[[Glu]] acg	gag

Please amend the Table 146 at page 466, as follows:

[Table 146]

Number	Mutated site	Change in amino acid sequence		Change in base sequence	
		Before mutation	After mutation	Before mutation	After mutation
No. 88d	171st position in β-subunit	Lys	[[aag]] Ala	[[Ala]] aag	gcg

Please amend the paragraph at page 481, line 2, as follows:

In a 500 ml Erlenmeyer flask with baffles, [[57]] 56 sets of a liquid LB medium of 100 ml each containing 40 μg/ml of ferric sulfate·heptahydrate and 10 μg/ml of cobalt chloride·hexahydrate were prepared and sterilized by autoclaving at 121°C for 20 minutes. To each medium, ampicillin was added to a final concentration of 100 μg/ml.

Please amend the paragraph at page 481, line 8, as follows:

~~Fifty-seven~~ Fifty-six types of transformants such as a transformant No. 0 obtained by transforming HB101 with pPT-DB1 and the following transformants obtained in Examples 89 to 117: No. 40, No. 40e, No. 40f, No. 42, No. 42a, No. 43, No. 44, No. 45, No. 46, No. 47, No. 48, No. 49, No. 50, No. 51, No. 52, No. 54, No. 55, No. 56, No. 57, No. 58, No. 59, No. 60, No. 61, No. 62, No. 63, No. 64, No. 65, No. 66, No. 67, No. 68, No. 69, No. 70, No. 71, No. 72, No. 73, No. 74, No. 75, No. 76, No. 77, No. 78, No. 79, No. 80, No. 81, No. 82, No. 83, No. 84, No. 85, No. 87, No. 88, No. 89, No. 90, No. 91, No. 92, No. 93, No. 94 and No. 95, were inoculated onto the above media such that one platinum loop of each of the transformant was inoculated on each of the 57 media. The cells were incubated therein at 37°C for about 20 hours with stirring at 130 rpm. Each of the transformants was separated from the resulting culture by centrifugation (5,000 G × 15 minutes). Subsequently, the separated transformants were resuspended in 50 ml of physiological saline, and then subjected to another centrifugation (5,000 G × 15 minutes), thereby each transformant being separated.

Please amend the paragraph at page 482, line 3, as follows:

0.1 g of each of the transformants were suspended in 20 ml of an aqueous solution (pH 7.0) of 50mM potassium phosphate, and then the suspensions were divided into 10 ml × 2 sets. Thus, two suspensions per each tranformant, that is, [[10]] 112 suspensions in total, were prepared. To one suspension of each transformant, 1 ml of acrylonitrile was added, and to the other suspension, methacrylonitrile was added. The suspensions were gently stirred at 20°C for 10 minutes to react.